Project JP 7706
Micro 7824

STORAGE OF MICROBIAL CULTURE MEDIA

J.E. Pagel and G.M. Hardy

MICROBIOLOGY SECTION
LABORATORY SERVICES BRANCH
MINISTRY OF THE ENVIRONMENT

JANUARY, 1979

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A. Introduction:

The storage of microbial culture media is common practice, necessitated by the inconvenience of time-consuming preparation procedures and sterilization processes. The shelf life of a prepared medium is affected by the type of medium and the storage conditions. Throughout any length of storage, culture media undergo physical and chemical changes which at some point begin to significantly alter their performance and efficiency. Some of the factors affecting the useful life of a medium include formulation, method of preparation, storage container, relative humidity, storage temperature, exposure to light and bacterial or fungal contamination (Martin, 1971; Difco Laboratories, 1975). The purpose of this project was to determine shelf lives for some of the more commonly used culture media in the Microbiology Section, by monitoring the effects of ageing on the appearance, quality and performance of these media.

B. Materials and Methods:

The culture media chosen for study were in most cases those employed daily in the Central Water Quality and Regional Laboratories. The choice of media was also intended to complement and expand on preliminary work done as part of an Experience '77 student project (Pagel and Hart, 1977). Table 1 lists the culture media, tube or plate size, dispensed volume and storage container. All media were prepared and dispensed according to the procedures described in the MOE "Handbook of Analytical Methods for Environmental Samples". Storage was at $4^{0}C$ in a walk-in Constant Temperature Refrigerator. Media were

.....2

monitored over a six month period for pH, volume, sterility, appearance and performance with positive and negative control cultures. The pH was measured by means of a Corning pH Meter for tubed media and a Beckman Instrument flat-bottom electrode for plated media. Volume was measured in duplicate for broth media by randomly selecting two tubes from a basket and using a graduated cylinder. Depth for the plated media was recorded in mm. Sterility was checked by incubating one tube or plate at 20°C for three days, and two tubes or plates at 35°C for the same time period. Observations were made daily and a description of any contaminating growth recorded. Overall appearance and medium consistency was described and any change with increased storage noted. Performance was measured using a positive and a negative control culture for each medium and these results are listed along with the expected reactions in Tables 2 to 13. Testing for these parameters was performed weekly for the first eight weeks and then monthly for the remaining four months.

C. Results:

The results of these tests are given in Tables 2 to 13. The pH measurement shown for each week is the arithmetic mean of two readings from two tubes or plates. Bacterial reactions are expressed as acid (A), gas (G), growth (Gr), no growth or visible reaction (-), clotting (Cl), pigment (P), fluorescence (F), and caseinase production (C). Other positive reactions (+) are explained in the Comments column. Sterility is recorded

.....3

as absence (-) or presence (Gr) of contamination and the type of contamination is described in the Comments column. The volume or depth of medium was measured using two tubes or plates and both readings are given (A and B). The overall appearance was recorded based on observations of the entire amount of medium stored and "as above" indicates that the appearance was the same as the previous recorded description (eg. in the case of PA broth an increase in the amount of precipitate was noted after three weeks of storage and this remained the same for the remainder of the experiment).

1. Presence-Absence Broth (Table 2)

The PA Broth supported acid and gas production from E.coli during the entire six month test period. The pH and volume decreases were negligible and there was no evidence of contamination. After three weeks of storage the normal amount of white precipitate present increased slightly and may have interfered with reading for growth not accompanied by acid production. (Note: The PA Broth was prepared with BBL MacConkey Broth as base).

2. Tryptic Soy Broth (Table 3)

In six months of storage, Tryptic Soy Broth continued to support good growth of both E. coli and S.faecalis stock cultures. Less turbidity was produced by S.faecalis but this was typical for this culture and did not decrease with storage of the medium. No appreciable pH change occurred and the decrease in volume noted was approximately one ml after six months. No change in overall appearance of the broth was observed.

.....4

3. Skim Milk Broth (Table 4)

Definite changes were observed in the tubes of Skim Milk Broth during the six months of storage. A rapid pH increase of 0.15 unit took place in the first week, and by week 24 the total increase was 0.45 unit. After five weeks of storage a grey zone was observed at the bottom of the stored, uninoculated tubes to the level of the iron nail. This zone of darkening increased to approximately half the volume level of the broth. These two changes were probably caused by ferric hydroxide ($Fe(OH)_3$) accumulating as a colloidal suspension around the iron nail. The C. perfringens culture produced strong " stormy fermentation" with acid, clot and gas production until the 24 week test when no clot was formed. The stock culture of Lactobacillus sp. produced no reaction as expected until week 12 when a slight gelling effect was noted. This evidence of protein breakdown was observed after 48 hours of incubation in week 16. As this reaction was not repeated in subsequent tests, it may have been caused by incubation of ageing medium rather than by the Lactobacillus sp. A decrease in volume of two ml was recorded over the six months. Sterility was checked by spread-plate onto Tryptic Soy Agar (Difco) and no contamination was observed.

4. Drake's Broth (Table 5)

No storage problems were evident in the Drake's Broth tubes. The pH fluctuated slightly but remained within the 7.6 ± 0.2 guidelines, and the volume fluctuated but was within approximately one ml in five months. The P. aeruginosa culture provided the same fluorescence and pale green pigment throughout the test period and Bacillus sp. gave no reaction although it

.....5

was able to grow. No problems with sterility were observed and the overall appearance of the uninoculated, stored medium remained the same.

5. m Enterococcus Agar (Tables 6 and 7)

Little difference was noted between storage effects on large and small plates of m Enterococcus Agar, although the latter were stored in the Gelman plate boxes. The pH measurements remained within the guidelines (7.2 ± 0.2) and there were no contamination problems observed. Streptococcus faecalis produced the same size and colour of colonies throughout the test period and S.aureus showed no growth. Little dehydration was evident in the small plates of m Enterococcus Agar; however the depth in the large plates decreased from approximately 2.5 to 3.0 mm to approximately 2.0 to 2.5 mm. The slight roughening of the surface of the agar after drying did not appear to affect the performance of the medium and did not seem to be related to length of storage.

6. MacConkey Agar (Table 8)

MacConkey Agar showed a slight decrease in pH in six months but remained within the acceptable limits of 7.1 ± 0.2. Escherichia coli produced pink (lactose positive) colonies in the first five weeks and slightly darker pink colonies in succeeding weeks. Staphylococcus aureus showed no growth throughout the test period and no contamination was observed. As in the case of large plates of m Enterococcus Agar, some dehydration occurred and the depth decreased from 2.5 - 3.0 mm to 2.0 - 2.5 mm. The overall appearance remained the same, although the dehydration may have been responsible for the apparently stronger lactose reaction by E.coli.

.....6

7. Tryptic Soy Agar (Table 9)

Little change in pH was observed for Tryptic Soy Agar. Both stock cultures showed typical growth even after six months of storage. Occasional contamination was noted, probably at a typical rate for non-selective media. In each case only one or two contaminating colonies were present. Little dehydration occurred and the overall appearance remained the same.

8. mPA Agar (Table 10)

The maximum storage period recommended for mPA Agar is 30 days. In the present study we observed little change in pH and no sterility problems. The P.aeruginosa culture produced the same size and colour of colonies throughout the storage period and A.hydrophila was unable to grow. Dehydration was genrally negligible until the 24 week observations; however the overall appearance was affected in week six by the appearance of fine white crystals on the surface of the agar. While these were well dispersed and did not seem to affect the bacterial reaction, the crystals were probably from one or more of the antibiotics and may have changed the inhibitory level of the medium if a natural sample had been tested.

9. Skim Milk Agar (Table 11)

The pH of this medium did not change appreciably in the storage period but did start at the upper end of the recommended range (6.4 ± 0.2). Contamination was noted in several plates in the first month of storage, but did not approach the extent of sterility problems noted with earlier preparation methods (Pagel, 1977). Throughout the storage interval, P.aeruginosa gave strong caseinase and pigment production,

.....7

but the fluorescence was weaker after eight weeks. Escherichia coli grew well with no pigment or caseinase production as expected. No dehydration was evident and the appearance of the uninoculated agar did not change.

10. Acetamide Agar (Table 12)

Acetamide Agar gave a slight increase in pH but stayed within the acceptable limits (6.8 $\pm$ 0.2). Utilization of acetamide by P. aeruginosa was strongly positive for the first four months but weaker after this amount of storage. In weeks 12 and 16, P. vesicularis, which is unable to utilize acetamide, appeared to show slight growth at the base of the slant. No alkalinity was present and slight carry over of nutrients during inoculation may have been responsible for this observation. The volume and appearance of this medium did not change during storage.

11. Nutrient Gelatin (with Yeast Extract) Agar

The pH of NGYE plates decreased slightly but remained within acceptable guidelines (6.8 $\pm$ 0.2). Aeromonas hydrophila grew and hydrolysed the gelatin, and E. coli grew with no hydrolysis throughout the test period. Mold contamination became a problem at 20 weeks of storage, and at the end of the six months, 12 of the remaining 42 plates were found to be contaminated at 4^{0}C. Little dehydration was evident in depth measurements although some wrinkling became apparent after three weeks of storage.

12. EC Broth

A separate eight week experiment was conducted to verify results in the Pagel and Hart report of 1977 which indicated a large (30 per cent) decrease in volume after six weeks of storage. Weight in grams was measured for 10 tubes of single-strength EC Broth in 16 x 150 mm metal capped tubes containing

a Durham gas tube. These tubes were stored at 4^0C and were weighed weekly. The average weight loss was 0.20 grams or approximately one per cent in eight weeks.

Volume in ml was measured using a 10 ml pipette, by randomly selecting 10 tubes each week. The average volume loss was 0.37 ml or approximately four per cent. No contamination problems were noted.

The discrepancy between this set of results compared to those obtained earlier is hard to explain and must be attributed either to differences in experimental design in the 1977 study or to error in measurement technique. Certainly the rate of dehydration observed in the present study is more consistent with results obtained for other broth media.

D. Conclusions and Recommendations:

Generally the results of this study confirmed those of previous work (Pagel and Hart, 1977); that is, the observed effects of prolonged storage are generally less than anticipated. Based on the observations tabled in this report, assuming similar storage conditions, and taking into account the fact that stressed cultures rather than stock cultures are involved, several recommendations can be made. In most cases the shelf life restrictions suggested in the report "Microbiology quality assurance programs" (1976) are supported by this study. The recommended storage periods of four weeks for Tryptic Soy Agar, Skim Milk Agar and NGYE Agar may be increased to six weeks based on the results of this study. Drake's Broth should store satisfactorily for 12 weeks while the shelf life for Acetamide Agar should be reduced to eight weeks.

E. Bibliography:

Difco Laboratories (1974). Quality Control of Culture Media. Difco Laboratories, Detroit, Michigan.

Martin, R.J. (1971). Storage of microbial culture media. Lab. Practice, 20 (8):653-656.

Ontario Ministry of the Environment. Laboratory Services Branch. (1976). Handbook of Analytical Methods for Environmental Samples.

Pagel, J.E. (1976) Microbiology quality assurance programs. Data Quality Report Series. Ontario Ministry of the Environment, 43 pp.

Pagel, J.E. and Hart, R. (1977). Experience '77 Student project - Quality assurance. Ontario Ministry of the Environment, 59 pp.

Table 1.

CULTURE MEDIA AND STORAGE CONDITIONS

Culture Medium	Tube/ Bottle	Plate	Dispensed Vol. (ml)	Storage Conditions
Presence-Absence (PA) Broth	8 oz.		78	caps tightened
Tryptic Soy Broth (Difco)	16x100 mm metal cap		10	test tube basket
Skim Milk Broth	18x150 mm metal cap		20	"
Drake's Broth	18x150 mm metal cap		10	"
m Enterococcus Agar (Difco)	7x47 mm round (Gelman)		4	Gelman plate box
	15x100 mm square		25	closed cakette
MacConkey Agar (Difco)		15x100 mm round	20	closed cakette
Tryptic Soy Agar (Difco)		15x100 mm round	20	closed cakette
mPA Agar		15x100 mm round	20	closed cakette
Skim Milk Agar		15x100 mm round	20	closed cakette
Acetamide Agar	16x100 mm screw cap		5	caps tightened
Nutrient Gelatin (with Yeast Extract) Agar (NGYE)		15x100 mm round	20	closed cakette

Table 2. STORAGE EFFECTS ON PA BROTH (8 oz.)

Week	pH	Bacterial Reactions E. coli (+)			Sterility 20°C			Sterility 35°C (duplicate)			Volume (ml)		Overall Appearance and Comments
		24h	48h	72h	24h	48h	72h	24h	48h	72h	A	B	
0	7.35	AG	AG	AG	-	-	-	-	-	-	73.0	73.5	transparent purple broth with slight precipitate at bottom
1	7.35	AG	AG	AG	-	-	-	-	-	-	74.5	74.5	as above
2	7.3	AG	AG	AG	-	-	-	-	-	-	73.0	74.0	as above
3	7.2	AG	AG	AG	-	-	-	-	-	-	73.0	73.0	slight increase in amount of precipitate
4	7.25	AG	AG	AG	-	-	-	-	-	-	73.0	73.0	as above
5	7.33	AG	AG	AG	-	-	-	-	-	-	73.5	73.0	as above
6	7.25	AG	AG	AG	-	-	-	-	-	-	72.5	73.5	as above
7	7.2	AG	AG	AG	-	-	-	-	-	-	73.5	72.5	as above
8	7.2	AG	AG	AG	-	-	-	-	-	-	73.5	74.0	as above
12	7.25	AG	AG	AG	-	-	-	-	-	-	73.5	74.0	as above
16	7.3	AG	AG	AG	-	-	-	-	-	-	73.0	73.0	as above
20	7.3	AG	AG	AG	-	-	-	-	-	-	74.0	72.0	as above
24	7.28	AG	AG	AG	-	-	-	-	-	-	72.0	72.0	as above

Expected pH is 7.4 ± 0.2
See text for further explanation
ND = not done

Table 3. STORAGE EFFECTS ON TRYPTIC SOY BROTH

Week	pH	Bacterial Reactions						Sterility						Volume (ml)		Overall Appearance and Comments
		E.coli (+)			S.faecalis (+)			20°C			35°C (duplicate)					
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	A	B	
0	7.35	+	+	+	+	+	+	-	-	-	-	-	-	10.0	9.5	S.faecalis typically produced weaker growth than E.coli after 24 hours of incubation
1	7.35	+	+	+	+	+	+	-	-	-	-	-	-	10.0	10.0	pale yellow transparent broth
2	7.35	+	+	+	+	+	+	-	-	-	-	-	-	10.0	9.75	as above
3	7.35	+	+	+	+	+	+	-	-	-	-	-	-	10.0	10.0	as above
4	7.4	+	+	+	+	+	+	-	-	-	-	-	-	9.0	9.0	as above
5	7.4	+	+	+	+	+	+	-	-	-	-	-	-	9.0	10.0	as above
6	7.35	+	+	+	+	+	+	-	-	-	-	-	-	10.0	9.5	as above
7	7.4	+	+	+	+	+	+	-	-	-	-	-	-	9.75	10.0	as above
8	7.35	+	+	+	+	+	+	-	-	-	-	-	-	10.0	9.5	as above
12	7.35	+	+	+	+	+	+	-	-	-	-	-	-	9.5	10.0	as above
16	7.45	+	+	+	+	+	+	-	-	-	-	-	-	8.5	9.5	as above
20	7.36	+	+	+	+	+	+	-	-	-	-	-	-	9.5	9.0	as above
24	7.35	+	+	+	+	+	+	-	-	-	-	-	-	9.0	9.0	as above

Expected pH is 7.3 ± 0.2

See Text for further explanation

Table 4. STORAGE EFFECTS ON SKIM MILK BROTH

Week	pH	Bacterial Reactions						Sterility						Volume (ml)		Overall Appearance and Comments
		C.perfringens			(+)Lactobacillus sp.(-)			20°C			35°C (duplicate)					
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	A	B	
0	6.4	-	ACIG	ACIG	-	-	-	-	-	-	-	-	-	20.0	19.0	light cream coloured broth; sterility checked by spread-plate on TSA
1	6.55	-	ACIG	ACIG	-	-	-	-	-	-	-	-	-	19.0	19.0	as above
2	6.57	-	ACIG	ACIG	-	-	-	-	-	-	-	-	-	18.5	19.0	as above
3	6.6	-	ACIG	ACIG	-	-	-	-	-	-	-	-	-	18.0	20.0	as above
4	6.6	-	ACIG	ACIG	-	-	-	-	-	-	-	-	-	18.0	18.5	as above
5	6.68	ACIG	ACIG	ACIG	-	-	-	-	-	-	-	-	-	18.5	18.0	bottom zone to height of nail is slightly greyish in <u>all</u> tubes stored
6	6.6	-	ACIG	ACIG	-	-	-	-	-	-	-	-	-	18.5	18.5	as above
7	6.65	-	ACIG	ACIG	-	-	-	-	-	-	-	-	-	18.0	18.5	as above
8	6.63	-	ACIG	ACIG	-	-	-	-	-	-	-	-	-	18.0	18.0	broth is greyish brown with bottom grey zone
12	6.7	-	ACIG	ACIG	-	-	-	-	-	-	-	-	-	19.0	19.0	as above; slight protein breakdown producing a gel effect after 96h in <u>Lactobacillus</u> tube
16	6.88	-	ACIG	ACIG	-	+	+	-	-	-	-	-	-	17.5	18.5	as above with bottom half darker; broth slightly gelled in <u>Lactobacillus</u> tube
20	6.83	-	ACIG	ACIG	-	-	-	-	-	-	-	-	-	19.0	18.5	overall appearance is as above
24	6.85	-	AG	AG	-	-	-	-	-	-	-	-	-	18.0	18.0	overall appearance is as above

Expected pH is 6.4 ± 0.2

See text for further explanation

Table 5. STORAGE EFFECTS ON DRAKE'S BROTH

Week	pH	Bacterial Reactions						Sterility						Volume (ml)		Overall Appearance and Comments
		P.aeruginosa (+)			Bacillus sp. (-)			20°C			35°C (duplicate)					
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	A	B	
0	7.45	GrPF	GrPF	GrPF	N D	N D	N D	-	-	-	-	-	-	11.0	11.0	clear, transparent broth; P.aeruginosa pigment is pale green
1	7.45	GrPF	GrPF	GrPF	Gr	Gr	Gr	-	-	-	-	-	-	11.0	11.0	as above
2	7.5	GrPF	GrPF	GrPF	Gr	Gr	Gr	-	-	-	-	-	-	10.0	10.0	as above
3	7.5	GrPF	GrPF	GrPF	Gr	Gr	Gr	-	-	-	-	-	-	10.0	10.5	as above
4	7.6	GrPF	GrPF	GrPF	Gr	Gr	Gr	-	-	-	-	-	-	9.0	9.0	as above
5	7.55	GrPF	GrPF	GrPF	Gr	Gr	Gr	-	-	-	-	-	-	10.0	10.5	as above
6	7.5	GrPF	GrPF	GrPF	Gr	Gr	Gr	-	-	-	-	-	-	10.0	10.0	as above
7	7.53	GrPF	GrPF	GrPF	Gr	Gr	Gr	-	-	-	-	-	-	10.0	10.5	as above
8	7.58	GrPF	GrPF	GrPF	Gr	Gr	Gr	-	-	-	-	-	-	10.0	10.0	as above; Bacillus sp. growth is weak
12	7.6	GrPF	GrPF	GrPF	Gr	Gr	Gr	-	-	-	-	-	-	9.0	10.0	as above
16	7.53	GrPF	GrPF	GrPF	Gr	Gr	Gr	-	-	-	-	-	-	10.0	10.0	as above
20	7.53	GrPF	GrPF	GrPF	Gr	Gr	Gr	-	-	-	-	-	-	10.0	10.0	as above
24	7.44	GrPF	GrPF	GrPF	Gr	Gr	Gr	-	-	-	-	-	-	9.0	9.0	as above

Expected pH is 7.6 ± 0.2

See text for further explanation

ND = not done

Table 6. STORAGE EFFECTS ON m ENTEROCOCCUS AGAR (Small plates)

Week	pH	Bacterial Reactions						Sterility						Depth (mm)		Overall Appearance and Comments
		S.faecalis (+)			S.aureus (-)			20°C			35°C (duplicate)					
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	A	B	
0	7.3	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	pale greyish-pink agar, S.aureus no growth, S.faecalis maroon colonies
1	7.2	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above; agar surface roughens after drying in laminar flow hood
2	7.2	+	+	+	-	-	-	-	-	-	-	-	-	2.0	1.5	as above
3	7.27	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above
4	7.3	+	+	+	-	-	-	-	-	-	-	-	-	1.5	2.0	as above
5	7.3	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above
6	7.23	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above
7	7.25	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above
8	7.2	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above
12	7.2	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above
16	7.25	+	+	+	-	-	-	-	-	-	-	-	-	1.5	2.0	as above
20	7.23	+	+	+	-	-	-	-	-	-	-	-	-	1.5	2.0	as above
24																insufficient plates for testing

Expected pH is 7.2 $\pm$ 0.2

See text for further explanation

Table 7. STORAGE EFFECTS ON m ENTEROCOCCUS AGAR (large plates)

Week	pH	Bacterial Reactions						Sterility						Depth (mm)		Overall Appearance and Comments
		S.faecalis (+)			S.aureus (-)			20°C			35°C (duplicate)					
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	A	B	
0	7.35	+	+	+	-	-	-	-	-	-	-	-	-	2.5	3.0	pale greyish-pink agar S.aureus no growth S.faecalis maroon colonies
1	7.2	+	+	+	-	-	-	-	-	-	-	-	-	3.0	3.0	as above
2	7.25	+	+	+	-	-	-	-	-	-	-	-	-	2.0	3.0	as above
3	7.25	+	+	+	-	-	-	-	-	-	-	-	-	1.5	2.0	as above
4	7.35	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above
5	7.23	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above
6	7.25	+	+	+	-	-	-	-	-	-	-	-	-	2.0	3.0	as above
7	7.25	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above
8	7.25	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above
12	7.3	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	slightly lumpy appearance
16	7.3	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.5	as above
20	7.25	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above
24	7.3	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.5	as above

Expected pH is 7.2 ± 0.2

See text for further explanation

Table 8. STORAGE EFFECTS ON MacCONKEY AGAR

Week	pH	Bacterial Reactions						Sterility						Depth (mm)		Overall Appearance and Comments
		E.coli (+)			S.aureus (-)			20°C			35°C (duplicate)					
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	A	B	
0	7.2	+	+	+	-	-	-	-	-	-	-	-	-	2.5	3.0	reddish-brown agar; E.coli produces pink (lactose +) colonies, S.aureus no growth
1	7.2	+	+	+	-	-	-	-	-	-	-	-	-	2.5	2.5	as above
2	7.2	+	+	+	-	-	-	-	-	-	-	-	-	2.5	2.5	as above
3	7.08	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.5	as above
4	7.1	+	+	+	-	-	-	-	-	-	-	-	-	2.0	3.0	as above
5	7.13	+	+	+	-	-	-	-	-	-	-	-	-	2.5	3.5	as above
6	7.08	+	+	+	-	-	-	-	-	-	-	-	-	3.0	2.0	as above
7	7.1	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above
8	7.1	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above
12	7.08	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above
16	7.13	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above
20	7.08	+	+	+	-	-	-	-	-	-	-	-	-	2.5	2.0	as above
24	6.95	+	+	+	-	-	-	-	-	-	-	-	-	2.5	2.0	as above

Expected pH is 7.1 ± 0.2

See text for further explanation

Table 9 STORAGE EFFECTS ON TRYPTIC SOY AGAR

Week	pH	Bacterial Reactions						Sterility						Depth (mm)		Overall Appearance and Comments
		E.coli (+)			S.faecalis (+)			20°C			35°C (duplicate)					
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	A	B	
0	7.3	+	+	+	+	+	+	-	-	-	-	-	-	4.0	3.75	S.faecalis typically produced weaker growth than E.coli after 24 hours of incubation
1	7.35	+	+	+	+	+	+	-	-	-	-	-	-	3.0	3.5	pale straw coloured agar
2	7.3	+	+	+	+	+	+	-	-	-	-	-	-	3.0	3.5	as above
3	7.33	+	+	+	+	+	+	-	-	-	-	Gr		3.5	3.5	as above; one surface bacterial colony on one 35°C sterility plate
4	7.4	+	+	+	+	+	+	-	-	-	-	-	-	3.0	3.5	as above
5	7.3	+	+	+	+	+	+	-	-	-	-	-	-	4.0	3.5	as above
6	7.34	+	+	+	+	+	+	-	-	-	-	-	-	3.0	3.5	as above
7	7.3	+	+	+	+	+	+	-	-	-	-	Gr		4.0	3.5	as above; two bacterial colonies on one 35°C sterility plate
8	7.3	+	+	+	+	+	+	-	-	-	-	-	-	3.5	4.0	as above
12	7.28	+	+	+	+	+	+	-	-	-	-	-	-	3.5	4.0	as above
16	7.23	+	+	+	+	+	+	-	-	-	-	-	-	3.5	4.0	as above
20	7.18	+	+	+	+	+	+	-	-	-	-	-	-	3.0	3.0	as above
24	7.2	+	+	+	+	+	+	-	-	Gr	-	-	-	3.0	4.0	as above; mold contamination on 20°C plate

Expected pH is 7.3 ± 0.2

See text for further explanation

Table 10. STORAGE EFFECTS ON mPA AGAR

Week	pH	Bacterial Reactions						Sterility						Depth (mm)		Overall Appearance and Comments
		P.aeruginosa (+)			A.hydrophila (-)			20°C			35°C (duplicate)					
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	A	B	
0	7.65	+	+	+	-	-	-	-	-	-	-	-	-	3.0	3.0	red transparent agar; P.aeruginosa produces grey-brown colonies, medium turns alkaline
1	7.6	+	+	+	-	-	-	-	-	-	-	-	-	3.0	3.0	A.hydrophila no growth
2	7.58	+	+	+	-	-	-	-	-	-	-	-	-	3.0	3.0	as above
3	7.58	+	+	+	-	-	-	-	-	-	-	-	-	3.0	3.0	as above
4	7.55	+	+	+	-	-	-	-	-	-	-	-	-	2.5	3.0	as above
5	7.6	+	+	+	-	-	-	-	-	-	-	-	-	3.0	2.5	as above
6	7.6	+	+	+	-	-	-	-	-	-	-	-	-	3.0	3.0	crystals (of antibiotics?) on surface; culture reactions stay the same
7	7.6	+	+	+	-	-	-	-	-	-	-	-	-	2.5	2.5	as above
8	7.55	+	+	+	-	-	-	-	-	-	-	-	-	2.0	3.0	as abpve
12	7.53	+	+	+	-	-	-	-	-	-	-	-	-	3.0	3.0	as above
16	7.6	+	+	+	-	-	-	-	-	-	-	-	-	3.0	3.0	as above
20	7.55	+	+	+	-	-	-	-	-	-	-	-	-	2.5	3.0	as above
24	7.6	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above

Expected pH is 7.6 ± 0.1

See text for further explanation

Table 11. STORAGE EFFECTS ON SKIM MILK AGAR

Week	pH	Bacterial Reactions						Sterility						Depth (mm)		Overall Appearance and Comments
		P.aeruginosa (+)			E. coli (-)			20°C			35°C (duplicate)					
		24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	A	B	
0	6.6	CPF	CPF	CPF	ND	ND	ND	-	-	-	-	-	Gr	2.0	2.5	smooth, white agar, P.aeruginosa pigment is green; E.coli produces large cream colonies, no pigment or fluorescence. Contamination in week 0,2 bacterial colonies on both plates.
1	6.65	CPF	CPF	CPF	ND	ND	ND	-	-	-	-	-	-	2.5	2.5	
2	6.6	CPF	CPF	CPF	Gr	Gr	Gr	-	-	-	-	-	-	2.0	2.5	
3	6.68	CPF	CPF	CPF	Gr	Gr	Gr	-	-	-	-	Gr		2.0	2.0	appearance as above; one contaminating colony present
4	6.7	CPF	CPF	CPF	Gr	Gr	Gr	-	-	-	-	Gr		2.5	3.0	appearance as above; bacterial contamination spreading growth
5	6.7	CPF	CPF	CPF	Gr	Gr	Gr	-	-	-	-	-	-	3.5	2.5	appearance as above
6	6.7	CPF	CPF	CPF	Gr	Gr	Gr	-	-	-	-	-	-	2.0	2.0	as above
7	6.65	CPF	CPF	CPF	Gr	Gr	Gr	-	-	-	-	-	-	2.0	2.5	as above
8	6.6	CPF	CPF	CPF	Gr	Gr	Gr	-	-	-	-	-	-	2.5	2.5	as above; fluorescence not as bright in pos. control
12	6.6	CPF	CPF	CPF	Gr	Gr	Gr	-	-	-	-	-	-	2.0	3.0	as above
16	6.7	CPF	CPF	CPF	Gr	Gr	Gr	-	-	-	-	-	-	2.5	3.0	as above
20	6.55	CPF	CPF	CPF	Gr	Gr	Gr	-	-	-	-	-	-	2.0	2.5	as above
24	6.6	CPF	CPF	CPF	Gr	Gr	Gr	-	-	-	-	-	-	2.0	3.0	as above

Expected pH is 6.4 ± 0.2

See text for further explanation

ND = not done

Table 12. STORAGE EFFECTS ON ACETAMIDE AGAR

Week	pH	Bacterial Reactions: P.aeruginosa (+) 24h	48h	72h	P.vesicularis (-) 24h	48h	72h	Sterility: 20°C 24h	48h	72h	35°C (duplicate) 24h	48h	72h	Volume (ml) A	B	Overall Appearance and Comments
0	6.7	+	+	+	-	-	-	-	-	-	-	-	-	4.5	4.5	pale orange agar, P.aeruginosa produces alkaline reaction, change to pink, P.vesicularis no growth.
1	6.85	+	+	+	-	-	-	-	-	-	-	-	-	4.5	4.5	as above
2	6.85	+	+	+	-	-	-	-	-	-	-	-	-	4.5	4.5	as above
3	6.85	+	+	+	-	-	-	-	-	-	-	-	-	4.5	4.0	as above
4	6.9	+	+	+	-	-	-	-	-	-	-	-	-	4.5	4.5	as above
5	6.85	+	+	+	-	-	-	-	-	-	-	-	-	4.5	5.0	as above
6	6.85	+	+	+	-	-	-	-	-	-	-	-	-	4.5	5.0	as above
7	6.9	+	+	+	-	-	-	-	-	-	-	-	-	4.5	5.0	as above
8	6.85	+	+	+	-	-	-	-	-	-	-	-	-	4.5	5.0	as above
12	6.83	+	+	+	$\bar{+}$	$\bar{+}$	$\bar{+}$	-	-	-	-	-	-	4.5	5.0	appearance as above; P.vesicularis appears to produce slight growth probably due to carry over of nutrients
16	6.8	+	+	+	$\bar{+}$	$\bar{+}$	$\bar{+}$	-	-	-	-	-	-	5.0	4.5	
20	6.84	$\pm$	$\pm$	$\pm$	-	-	-	-	-	-	-	-	-	4.5	5.0	as above, positive reaction is weak
24	6.86	$\pm$	$\pm$	$\pm$	-	-	-	-	-	-	-	-	-	4.0	4.5	as above, positive reaction is weak

Expected pH is 6.8 ± 0.2

See text for further explanation

Table 13. STORAGE EFFECTS ON NGYE AGAR

Week	pH	Bacterial Reactions: A.hydrophila (+) 24h	48h	72h	E.coli (-) 24h	48h	72h	Sterility: 20°C 24h	48h	72h	35°C (duplicate) 24h	48h	72h	Depth (mm) A	B	Overall Appearance and Comments
0	7.0	+	+	+	-	-	-	-	-	-	-	-	-	3.0	3.0	grey-yellow agar, slightly wrinkled surface after drying; A.hydrophila growth plus gelatinase production; E.coli growth, no gelatinase production
1	6.85	+	+	+	-	-	-	-	-	-	-	-	-	3.0	3.0	
2	6.83	+	+	+	-	-	-	-	-	-	-	-	-	3.0	3.0	as above
3	6.88	+	+	+	-	-	-	-	-	-	-	-	-	3.0	3.0	as above with more surface wrinkling
4	6.9	+	+	+	-	-	-	-	-	-	-	-	-	3.5	2.5	appearance as above
5	6.9	+	+	+	-	-	-	-	-	-	-	-	-	3.5	3.0	as above
6	6.88	+	+	+	-	-	-	-	-	-	-	-	-	2.5	3.0	as above
7	6.83	+	+	+	-	-	-	-	-	-	-	-	-	2.0	3.0	as above
8	6.8	+	+	+	-	-	-	-	-	-	-	-	-	2.0	2.0	as above
12	6.85	+	+	+	-	-	-	-	-	-	-	-	-	3.0	3.0	as above
16	6.85	+	+	+	-	-	-	-	-	-	-	-	-	2.5	3.0	as above
20	6.83	+	+	+	-	-	-	-	-	Gr	-	-	-	2.0	3.0	as above; mold contamination on 20°C plate
24	6.9	+	+	+	-	-	-	-	-	-	-	-	Gr	3.0	3.0	as above; mold contamination on 35°C plate

Expected pH is 6.8 ± 0.2
See text for further explanation

(11931)
MOE/STO/AOCJ

MOE/STO/AOCJ
Pagel, J.E.
Storage of microbial
culture media aocj
1979 c.1 a aa